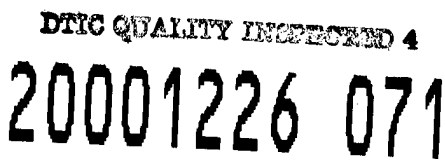


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# Histopathologic Changes in the Brain, Heart, and Skeletal Muscle of Rhesus Macaques, Ten Days after Exposure to Soman (an Organophosphorus Nerve Agent)

James O. Britt, Jr., Jamie L. Martin, Carlin V. Okerberg, and Edward John Dick, Jr.

**Background and Purpose:** Soman, an organophosphorus, anticholinergic, chemical warfare nerve agent, is studied at few research facilities, and there have been few pathologic studies of soman-exposed primates. We describe the brain, heart, and skeletal muscle lesions, review lesions described in literature, and discuss possible pharmacologic mechanisms for soman-induced neuron necrosis.

**Methods:** In this retrospective, histopathologic study, records were obtained for 36 rhesus macaques (*Macaca mulatta*) that were euthanized 10 days after soman exposure, from a larger group of 103 monkeys that were exposed to soman and used for pharmacologic and lethality studies.

**Results:** Brain lesions were seen in 9 of 15 animals that convulsed and in only 1 of 21 that did not convulse. The brain lesions in our primates were limited to the hippocampus, amygdala, and thalamus (of one animal), and consisted of neuron necrosis and dropout, spongiosis, gliosis, astrocytosis, and vascularization. Heart lesions consisted of myocardial degeneration and necrosis. Three animals had brain and heart lesions, 7 had brain lesions only, and 3 had heart lesions only. Skeletal muscle lesions, although minimal to mild, were in most of the animals, whether they had convulsed, but most had muscular tremors. These lesions were in the biceps brachii (11 of 22 monkeys), anterior tibialis (8/22), biceps femoris (7/22), flexor carpi radialis (5/22), gastrocnemius (3/22), and diaphragm (1/22). The limited literature on soman lesions in primate brain and heart, and the limited information on skeletal muscle lesions, is reviewed.

**Conclusions:** Brain lesions were not as wide-spread as reported in other studies of primates and rodents, and were significantly associated with convulsions. Unlike other studies using rodents, we observed poor correlation between heart and brain lesions; thus, a single hypothesis to explain the pathogenesis for the brain and heart lesions may be difficult to establish.

A review of the literature indicates that soman may not cause its debilitating neurologic effects by (or solely by) cholinergic seizure activity or direct, acute neuron toxicity. A secondary, non-cholinergic, neurotoxic mechanism may exist. A theory for continuing neuron necrosis, even though the acute cholinergic phase only lasts for about an hour or less in other experimental animals, is discussed. As this cholinergic surge subsides, secondary neurotransmitters may be recruited that either potentiate excitation of neurons, or alter the inhibitory substances or the sensitivity of neurons to normal activities of neurotransmitters.

Research on the nerve agent soman (pinacolyl methylphosphonofluoridate) is conducted at few facilities. This Institute is investigating the mode of action of soman in the search for more effective antidotes and pretreatments. This research is not classified, but is often reported in technical bulletins of limited distribution. Few studies have documented the pathologic effects of soman in primates, especially the lesions seen as long as 10 days after exposure. This study adds to the knowledge of the longer-term lesions.

Soman is a lethal organophosphorus nerve agent that binds irreversibly to acetylcholinesterase (AChE) at the central and

peripheral sites to cause buildup of the neurotransmitter acetylcholine (ACh) and sustained activation of ACh receptors (1, 2). This initiates rapid progression of miosis, hypersecretions, muscular fasciculation and tremors, seizures, convulsions, and death in laboratory rodents, nonhuman primates, and man (1, 3, 4). Experimental animals that have seizures for more than 20 to 40 minutes and live for one to several hours or longer develop neuronal necrosis (5). A review of a limited number of previous studies of soman toxicity in nonhuman primates (4, 6, 7) documented necrotizing brain lesions in the hippocampus, entorhinal cortex, frontal cortex, amygdaloid complex, caudate nuclei, and thalamus, with a few lesions in the pons, medulla, and cerebellum of animals that survived 2 to 7 days after exposure. The neural lesions were more severe in the monkeys that had convulsed.

In another 3-week study (8), soman-exposed cynomolgus monkeys (*Macaca fascicularis*) were treated with atropine/pralidoxime/diazepam or atropine/HI-6/prodiazepam, but had convulsions that subsided within 2 hours. They had decreased numbers of neurons in the cerebral cortex and shrinking and necrosis of neurons in the Purkinje cell layer. Also, neuronal necrosis was seen in the frontal cortex in one animal and in the hippocampus in another (8). The latter animal also had spongiosis in the pyriform cortex.

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Oximes such as pralidoxime chloride (2-PAM) and HI-6 can reactivate soman-phosphorylated AChE if given prior to aging of AChE (8). The lethality of soman has been documented to be decreased by pre-exposure treatment with pyridostigmine, a carbamate that reversibly binds with AChE and prevents the irreversible binding (aging) by soman (9), and postexposure treatment with atropine, 2-PAM, and diazepam, an anticonvulsant and antiepileptic agent (1). This triad protocol is the current U.S. military policy for personnel protection and treatment (1, 10, 11).

There are few reviews of soman lesions in primates. We report the histologic findings in the brain, heart, and skeletal muscles of rhesus macaques that survived 10 days after they were exposed to soman. These monkeys were used in an unpublished study on the minimal effective dose (MED) of pyridostigmine with soman (12).

## Materials and Methods

One hundred three male rhesus macaques (*Macaca mulatta*) were used in a five-phase study of soman and various treatment and pretreatment modalities (12), with the primary objective of determining the MED of pyridostigmine and the associated RBC AChE inhibition level to protect against soman. The MED of pyridostigmine was defined as the minimal dose that provided a 95% survival rate in monkeys injected with 5 times the soman dose that should be lethal to 50% of the monkeys that were treated with single doses of atropine (0.4 mg/kg of body weight, IM) and 2-PAM (25.7 mg/kg, IM). These experiments were conducted in stage-wise fashion to limit the number of monkeys to the minimum necessary to achieve statistically valid results.

The monkeys were subadult to adult, of unknown age and weighing 4 to 9 kg, and were quarantined for 1.5 months. They were housed individually in 24 x 26 x 36 inch stainless-steel cages with an automatic watering system and fluorescent lighting with a 12/12-hour light/dark cycle. The temperature was maintained at 18.3 to 28.9°C and the humidity at 30 to 70% with 10 to 15 air changes/h. The diet was commercial monkey biscuits and periodic fresh fruit and primate treats. Blood samples were taken for hematologic and biochemical analyses, and 2 fecal samples for internal parasite examination were collected 1 month apart. Tests for tuberculosis were performed by injecting tuberculin into the palpebral skin 3 times at 2- to 3-week intervals. Blood variables were within normal limits, internal parasites were not found, and results for all tuberculosis tests were negative.

Soman (U.S. Army Medical Institute for Chemical Defense, Aberdeen Proving Ground, MD) was given intramuscularly as a single dose in the right posterior tibial area in the region of the gastrocnemius muscle and, when given by injection, pyridostigmine bromide (Hoffmann-LaRoche, Inc., Nutley, NJ) was given once intramuscularly in the right lateral quadriceps 45 minutes before soman. When pyridostigmine bromide (Mestinon syrup, Hoffmann-LaRoche, from ICN Pharmaceuticals, Irvine, CA) was given orally, a single dose of 40 µg/kg was given by intragastric tube 150 minutes before soman challenge. When given, atropine (Survival Technology, Inc., Philadelphia, PA; 0.4 mg/kg, IM), 2-PAM (Protopam, Ayerst, Rouses Point, NY; 25.7 mg/kg, IM), and diazepam (Valium, Steris Laboratories, Inc., Phoenix, AZ; 0.1 mg/kg) were injected once in separate sites in the left quadriceps femoris muscle one minute after

soman challenge. The monkeys were continually monitored for tremors, convulsions, salivation, miosis, mydriasis, prostration, and death for 2 to 6 hours, depending on the study phase, then every 2 to 6 hours for 10 days.

The 36 monkeys that survived 10 days after soman exposure were sedated with ketamine (Vetalar, Ft. Dodge, IA; 10 mg/kg, IM), terminally anesthetized with sodium pentobarbital (Ft. Dodge Co., Ft. Dodge, IA; approx. 25 mg/kg, IV) to effect, and perfused with 1 L of heparinized saline, then 3 L of neutral-buffered 10% formalin, by use of a 2-inch blunt needle inserted into the left ventricle and the aorta while simultaneously being exsanguinated by an incision in a femoral vein. Twenty-four of these monkeys (group 1) had been pre-treated with varying doses of pyridostigmine, challenged with 32.5 µg/kg soman IM (one was given 60.0 µg/kg), and then further treated with 0.4 mg/kg atropine and 0.1 mg/kg 2-PAM (Table 1). One of these 24 monkeys was also given 0.1 mg of diazepam/kg, IM. Fifteen of these group-1 monkeys had convulsions and 9 did not; all 24 had muscular tremors.

Twelve more animals that survived 10 days had been given a lower dose of soman (4.5 to 15 µg/kg) and did not receive pyridostigmine (group 2). These were either not further treated or were given atropine/2-PAM (IM) or atropine/2-PAM/diazepam, using the same dosages as for those given the group-1 animals (Table 1). None of the group-2 animals had convulsions. Because there was a limited number of animals in each treatment subgroup, we chose to compare the lesions by subgrouping the animals by whether they had convulsions, regardless of the original treatment group.

The following tissues from group-1 animals were collected in formalin: heart (sinoatrial node [SA] area, left ventricle, right ventricle, interventricular septum, bundle of His, and left atrium), thoracic part of the spinal cord, peripheral nerves (phrenic, sciatic, peroneal, tibial, brachial plexus, and median nerves), skeletal muscle (diaphragm, biceps femoris, biceps brachii, anterior tibialis, gastrocnemius, and flexor carpi radialis), and brain. The formalin-fixed brain was processed by removing the brainstem with the cerebellum just anterior to the pons. The cerebellum was removed by a single horizontal cut between it and the brainstem, and 0.4-cm serial sections were continued through the cerebellum, parallel to this initial cut. The brainstem was then serially cross-sectioned every 0.4 cm parallel to the first cut at the pons. The cerebrum was serially cross-sectioned at 1.0 cm while resting on its dorsum. Thinner sections were made of desired areas at 0.4 cm and were placed in cassettes for routine processing, paraffin embedding, and sectioning at 6-µm thickness for routine H&E staining. The brain sections examined were: pituitary gland, frontal cortex, caudate, amygdala, pyriform cortex, hippocampus, thalamus, midbrain, pons, medulla, and cerebellum.

Only brain and heart were examined histologically in group-2 animals. Microscopic lesions (Table 1) in the brain, heart, and skeletal muscle were scored independently by 2 veterinary pathologists who were unaware of the treatment or convulsion information in advance, and initial scoring discrepancies were resolved by mutual re-evaluation. The score was by severity in the tissue or area of the brain examined: 0 = no lesions; 1 = minimal (1 to 10% of the tissue section affected); 2 = mild (11 to 25%); 3 = moderate (26 to 45%); 4 = severe (> 45%) (5). Because the animals with affected brains had lesions either limited to

Table 1. Soman lesions associated with pretreatment, treatment, and convulsions

Monkey # Group 1	Soman ( $\mu\text{g/kg}$ , IM)	PYR ( $\mu\text{g/kg}$ )	Tx	Convulsions	Histopathologic score*		
					Brain	Heart <sup>†</sup>	Muscle <sup>‡</sup>
1	32.5	4.0	A/2	Yes	0	0	0
2	32.5	4.0	A/2	Yes	4	0	1 <sup>c</sup>
3	32.5	4.0	A/2	Yes	4	0	2 <sup>b,fc</sup>
4	32.5	4.0	A/2	Yes	1	0	2 <sup>ce</sup>
5	32.5	4.0	A/2	Yes	1	0	2 <sup>c,d,e,f</sup>
6	32.5	8.4	A/2	Yes	0	0	0
7	32.5	8.4	A/2	Yes	0	0	0
8	32.5	8.4	A/2	Yes	4	2	2 <sup>b,c</sup>
9	32.5	24.0	A/2	Yes	0	0	1 <sup>c</sup>
10	32.5	24.0	A/2	Yes	1	0	2 <sup>c,f,b,d,e</sup>
11	32.5	24.0	A/2	Yes	4	0	1 <sup>c</sup>
12	32.5	40.0 (oral)	A/2	Yes	4	1	1 <sup>b,c,f</sup>
13	32.5	40.0 (oral)	A/2	Yes	0	1	0
14	32.5	40.0 (oral)	A/2	Yes	4	1	1 <sup>b,c,d,f</sup>
15	60.0	40.0 (oral)	A/2/D	Yes	0	0	1 <sup>e,f</sup>
16	32.5	8.4	A/2	No	0	1	1 <sup>d</sup>
17	32.5	8.4	A/2	No	0	0	1 <sup>a,f</sup>
18	32.5	24.0	A/2	No	0	0	1 <sup>f</sup>
19	32.5	4.0	A/2	No	0	0	1 <sup>b,c,e</sup>
20	32.5	8.4	A/2	No	0	0	0
21	32.5	8.4	A/2	No	4	0	1 <sup>b,c</sup>
22	32.5	8.4	A/2	No	0	ND	ND
23	32.5	40.0 (oral)	A/2	No	0	3	0
24	32.5	40.0 (oral)	A/2	No	0	ND	ND
Group 2							
25	4.5	0	None	No	0	ND	ND
26	5.3	0	None	No	0	ND	ND
27	5.5	0	A/2	No	0	ND	ND
28	7.0	0	A/2	No	0	ND	ND
29	7.3	0	A/2/D	No	0	ND	ND
30	7.5	0	A/2	No	0	ND	ND
31	8.0	0	A/2	No	0	ND	ND
32	8.5	0	A/2	No	0	ND	ND
33	9.2	0	A/2	No	0	ND	ND
34	10.0	0	A/2/D	No	0	ND	ND
35	15.0	0	A/2	No	0	ND	ND
36	20.0	0	A/2	No	0	ND	ND

\*Histopathologic scores (severity): 0 = no lesion; 1 = minimal; 2 = mild; 3 = moderate; 4 = severe, and ND = not done.

<sup>†</sup>Heart lesion sites and scores: sinoatrial node area (SA); right ventricle (RV); interventricular septum (VS); bundle of His (BH); left atrium (LA); left ventricle (LV). Monkey 8: SA=1; RV=0; VS=1; BH=1; LA=1; LV=2. Monkey 12: SA=1; RV=0; VS=0; BH=0; LA=1; LV=0. Monkey 13: SA=0; RV=0; VS=1; BH=0; LA=0; LV=0. Monkey 14: SA=1; RV=0; VS=1; BH=1; LA=0; LV=1. Monkey 16: SA=0; RV=0; VS=1; BH= not done; LA=not done; LV=1. Monkey 23: SA=not done; RV=0; VS=4; BH=not done; LA=not done; LV=1.

<sup>‡</sup>The letters identify the muscle group. When separated by a semicolon, the first muscle(s) had 2+ lesions and the second group of muscle(s) had 1+ lesions. a = diaphragm; b = biceps femoris; c = biceps brachii; d = gastrocnemius; e = flexor carpi radialis; and f = anterior tibialis.

A = Atropine (0.4 mg/kg); 2 = 2-PAM (25.7 mg/kg); D = diazepam (0.1 mg/kg); PYR = pyridostigmine (IM except as noted); Tx = treatment.

the hippocampus, or in the hippocampus with equal or lesser lesions in the amygdala or thalamus, the histologic score for the brain was based on the hippocampus section. Hippocampus sections from two animals with severe lesions and two from animals without microscopic lesions were also stained by use of cresyl violet and immunoperoxidase techniques for glial fibrillary acidic protein (GFAP), neurofilament protein (NFP), and the macrophage markers CD68 and lysozyme.

The heart and muscle lesions were scored in the section with the most severe lesions on the basis of percentage of myofibers affected. The statistical significance within group-1 monkeys was calculated by presence of brain, skeletal muscle, and heart lesions in the convulsing and non-convulsing subgroups, using a Kendall's Tau-c test.

## Results

**Group 1:** Appreciable gross necropsy lesions were not evident. Microscopically, 6 of the 15 animals that convulsed had severe brain lesions and three more had minimal lesions; only six had normal brains (Table 1). Development of convulsions was significantly associated with brain lesions in group-1 monkeys ( $P < 0.05$ ). Eleven monkeys that convulsed had normal

hearts (although six of these had brain lesions). Three had minimal heart lesions (two with severe brain lesions and one with normal brain), and one animal that convulsed had mild heart lesions with severe brain lesions. Of these four animals with heart lesions, two had multifocal myofiber degeneration and necrosis in the bundle of His region, with similar myofiber degeneration and necrosis and mild lymphocytic inflammation in the myocardium of the left ventricle (Figure 1). One of these two animals also had mild myofiber degeneration in the interventricular septum. The third animal had moderate focal necrosis in the left atrium and SA node area, and the fourth had localized mild myofiber loss and edema in the interventricular septum, without fibrosis.

Of the nine monkeys that did not convulse, only one had brain lesions but they were severe in the hippocampus. Although it was not observed to have convulsions, this animal had tremors for 144 hours. Mean duration was 3.74 hours for its treatment group in the clinical trial (12) and it had miosis for the entire 10-day observation period (mean, 1.35 hours). This monkey and four others that didn't convulse had normal hearts. The sixth had minimal heart lesions characterized by localized interventricular septal necrosis of individual myofibers. The

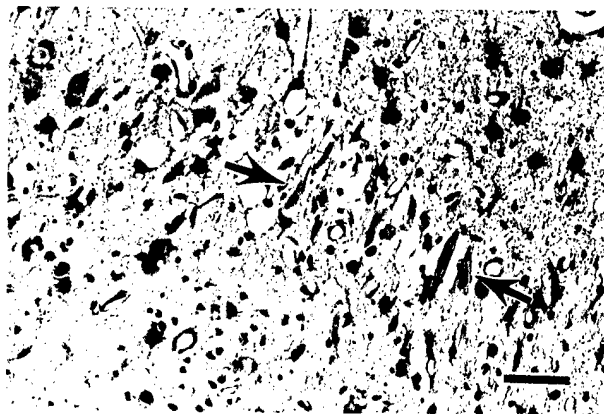


**Figure 1.** Photomicrograph of a section of the left ventricle from monkey 8. Notice acute cardiac myofiber necrosis (arrows) with early fibroplasia and mild heart lesions overall. H&E stain; bar = 50  $\mu$ m.

seventh had moderate interventricular septal necrosis and fibrosis, and had the most severe heart lesions of either the convulsion or nonconvulsion groups. The heart was not available for the other two non-convulsive animals.

Due to a specimen-collecting error, the amygdala was present for only three brains. Of the animals with convulsions, two had severe lesions in the hippocampus and amygdala whereas a third animal had normal amygdala with severe hippocampal lesions. The brain lesions were either minimal or severe and were limited to the hippocampus (and amygdala when present in two animals), except for one animal that also had mild lesions in the thalamus. The single animal with mild thalamic lesions also had severe hippocampal lesions. Minimal lesions in the hippocampus were characterized by one or more of the following changes: mild gliosis within the neuronal areas (pyramidal cell zone); increased microvascular prominence (with or without slight perivascular lymphocytic inflammation or edema); mild neuropil spongiosis; and, in one animal only, acute necrosis and phagocytosis of rare neurons. The mild thalamic lesion in the last animal was composed of a unilateral focus of glial cell proliferation with some intact neurons and apparent loss of other neurons. Acute neuron necrosis was not seen in the thalamus.

The severe lesions in the hippocampus were characterized by pyramidal layer spongiosis, gliosis, and loss of neurons in the CA1 (cornu Ammonis or Ammon's horn), CA3, and CA4 regions with relative sparing in CA2, and increased vascularity. Many of the remaining neurons were undergoing necrosis, characterized by cytoplasmic hypereosinophilia, with and without retention of the neuronal nuclei, contraction, or elongation and dropout (Figure 2). When the nuclei were still present in the degenerating neurons, they first appeared shrunken and more basophilic and had lost their delicate chromatin pattern and nucleolus (pyknosis). The end-stage degenerating neurons had increasingly pale eosinophilia of both the nucleus and cytoplasm. In the most necrotic pyramidal cell zones, most of the remaining neuron-like cells with large round nuclei with basophilic stippling and a prominent nucleolus stained strongly positive for GFAP in their perinuclear and more distant angular cytoplasm, indicating that these were reactive astrocytes. The GFAP-positive astrocytes were also increased in size and numbers throughout the molecular layer of the hippocampus. A few individual small hyperchromatic cells with minimal cytoplasm



**Figure 2.** Photomicrograph of a section of brain from monkey 11. Notice severe lesion in the hippocampus showing necrosis of most of the pyramidal neurons with pyknotic nuclei and hypereosinophilic cytoplasm (arrows), spongiform degeneration, and mild microgliosis, CA1, near junction with CA2. H&E stain; bar = 50  $\mu$ m.

(glial cells or lymphocytes) and rare neutrophils were adjacent to necrotic pyramidal neurons (neuronophagia). The NFP stain detected nearly complete loss of the normally plentiful, delicate axonal fibers throughout the spongiform portions of the pyramidal layer lesions and a moderate decrease in NSF-staining axons in the adjacent molecular layer. The cresyl violet stain was not helpful in distinguishing neurons from reactive cells, and the CD68 and lysozyme immunoperoxidase stains did not document macrophage lineage cells in the lesions.

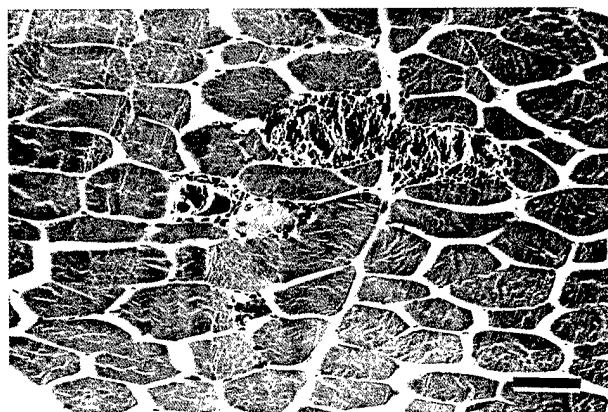
Spongiform change and loss of most neurons with many acutely necrotic neurons and diffuse gliosis characterized the severe lesions in the amygdala. Other regions of the brain, such as the frontal cortex, entorhinal cortex, pyriform cortex, and caudate, which have been reported to manifest soman lesions (4), were unremarkable in these monkeys.

Eleven of 15 animals with convulsions had muscle lesions (6, minimal; 5, mild); four animals had no muscle lesions (Table 1). Muscles of seven animals without convulsions were examined, and six animals had lesions (all minimal). Skeletal muscle lesions were characterized by multiple foci of myofiber degeneration/necrosis and hypercellularity due to proliferation of mononucleated and multinucleated sarcolemmal cells (that we judged to be consistent with a 10-day duration), but no leukocytic inflammation. Most of these degenerative/regenerative foci had mild cytoplasmic basophilia and some had mineralization within the sarcoplasm of the affected myofibers (Figure 3). Only one to three contiguous myofibers in the typical focus were affected. The most commonly affected muscles were the biceps brachii (12/22) and the anterior tibialis (8/22) muscles. The biceps femoris (7/22), flexor carpi radialis (5/22) gastrocnemius (4/22), and diaphragm (1/22) muscles were occasionally affected. Lesions were not found in the thoracic part of the spinal cord or peripheral nerves.

**Group 2:** None of the brains or heart sections in group-2 monkeys exposed to the lower dose of soman had histopathologic changes.

## Discussion

The finding of neuron necrosis being significantly associated with convulsions is consistent with findings in other primate (4)



**Figure 3.** Photomicrograph of a section of biceps brachii muscle from monkey 5 that had mild muscle lesions overall. Notice foci of myofiber degeneration with mineralization and multinucleated cells. H&E stain; bar = 100  $\mu$ m.

and rodent (5) studies. Brain lesions generally do not develop with < 45 minutes of convulsions (1). Transitory hypoxia has been proposed as an explanation for neuronal necrosis (13), but those authors also documented apparent ultrastructural neuronal degeneration beginning up to 72 hours after exposure to soman. The hypoxia theory would be consistent with the high oxygen demands of the hippocampus, but cerebral blood flow is actually increased during these seizures (14). We found that necrotic hippocampal neurons were still present 10 days after exposure, even though convulsions were limited to the first hour. Other neuron-dense areas, such as the cerebral cortex, would likely be affected also if transitory hypoxia (or hypoglycemia) was the sole mechanism of neuron necrosis.

Necrotic neurons in humans have been documented from 7 to at least 27 days after a single ischemic event (15). These longer-lasting necrotic neurons were called "neuronal ghosts" and correspond to the fading eosinophilic neurons without nuclei that we saw in some severe lesions. Eosinophilic neurons with residual pyknotic nuclei were gone by 10 to 13 days in that study, which correlates with our results of a combination of degenerating neurons with and without nuclei at 10 days after injury. Neuronal necrosis after exposure to soman is probably not due totally to soman-induced ACh buildup. In rats, seizures are successfully treated with anticholinergic drugs (atropine and scopolamine) up to 5 to 10 minutes after they begin, then become refractory to anticholinergic therapy after 40 minutes (1, 10).

Secondary neuropharmacologic events that occur after initial ACh stimulation would help explain this resistance to anticholinergic therapy and the neuron necrosis. Because seizures become completely refractory to anticholinergic therapy, it has been postulated that non-cholinergic neurotransmitters, such as glutamate, an excitatory amino acid, are recruited (10, 16). Thus, glutamate receptors may continue to be overstimulated after initial cholinergic activity has subsided (10, 16) causing intracellular calcium ion influx or other intracellular biochemical changes that are eventually toxic to neurons (5, 16). Also, results of *in vitro* studies indicate that glutamate, not soman, is directly toxic to neurons (17).

Regional and species variations in the neurotoxicity of soman might be explained by variation in the types of neurotransmitters present and recruited. For example, the primary excitatory

synapses in the hippocampus are glutamatergic (18). In rat studies, nitric oxide (NO) or drugs that deliver it to the brain in the proper redox state (14) and other N-methyl-D-aspartate (NMDA) antagonists (dizocilpine and trienylphenylcyclidine) have been documented to be neuroprotective, probably by blocking NMDA-glutamate receptors (2, 14). A recent report indicated that soman-intoxicated monkeys were completely protected from neurotoxicosis when gacyclidine, an experimental antiglutamatergic compound was given 45 minutes after intoxication was induced (19).

Neuron necrosis in this study might be explained by mechanisms of neurotoxicity other than continued absolute increase in excitotoxins. Remaining denervated neurons can become hypersensitive to normal activity of ACh or other transmitters (20), possibly by an increase in surface receptors in these isolated neurons. Also, denervation can lead to greatly decreased sensitivity to inhibitory neurotransmitters, such as  $\gamma$ -aminobutyric acid (GABA) (21).

It is unclear why the lesions in the affected brains were either minimal or severe. Perhaps there is a threshold level above which toxicosis rapidly becomes more severe. Inhibitory neurotransmitters, such as GABA, may actually increase in the brain after soman exposure, but inhibition may become ineffective when the amount of excitotoxic transmitters is overwhelming (22). Also, the inhibitory amino acid glycine is depleted in one model of hippocampal excitotoxicosis in rats and the brain can be protected by oral supplementation with glycine (23).

Only one animal that did not convulse had brain lesions, but its lesions were severe. This animal had prolonged tremors and miosis. There are no reliable behavioral changes to determine whether monkeys are having seizures (24). Convulsions in these monkeys were used as a clinical marker for seizure activity, but this animal may have had subclinical epileptiform seizures without muscular convulsions. Electroencephalographic studies are necessary to more accurately record seizure activity in the absence of clinical convulsions (2).

Similar to those in previous studies in guinea pigs (25), lesions in our monkeys were virtually limited to the hippocampus and amygdala; thus, emphasis should be placed on these regions in future primate studies. In this study, lesion development in the primate brain did not seem to be as sensitive an indicator of soman-induced damage as did lesion development described in rat brains. The latter have more broadly distributed brain lesions (pyriform cortex, amygdala, hippocampus, thalamus, cerebral cortex, and caudate/putamen, in decreasing frequency) (5). It is possible that minimal lesions or neuron loss without persistent gliosis or spongiosis might not have been detected after 10 days in our study without using morphometric analysis of specific regions.

Heart lesions associated with soman exposure have been postulated to be caused by a central neurogenic mechanism that may cause either adrenergic or cholinergic stimulation (3, 5, 26). The cardiac damage in rats reportedly develops more rapidly than does neural damage, and is not consistently associated with seizures (5). This primate study does not confirm that the heart is more sensitive to soman-induced damage than is the brain. There was poor correlation between heart damage and brain damage in our animals, and difference in heart lesions between the convulsant and non-convulsant subgroups of group-1 animals was not significant (Table 2). Some animals had

**Table 2.** Average lesion score for group-1 animals

Monkeys	Brain	Heart	Muscles
Convulsing (n=15)	1.80	0.33	1.06
Non-convulsing (n=9)	0.44	0.57	0.71

heart lesions without brain lesions, but others had severe brain lesions without heart lesions. Another primate study did not indicate heart lesions in animals with soman-induced neurotoxicosis (8). The temporal sequence and relationship of the brain and heart lesions remains to be determined, and pathogenesis of the heart lesions remains unclear. In our animals, there was not a statistical correlation between heart (and skeletal muscle) lesions in group-1 animals and the presence or absence of convulsions.

Only one other primate study (27) indicated skeletal muscle lesions after soman exposure. The report of that study did not describe the muscle lesions in detail, but documented that 3 of 13 soman exposed cynomolgus monkeys had mild, multifocal, myofiber necrosis or foci of regeneration by 48 hours. One study of rats indicated necrosis in a minority of myofibers in muscle bundles after soman exposure (28); the lesions resolved by 7 days and were present only in muscles with fasciculations. The muscles were protected from fasciculations and necrosis by atropine pretreatment, although AChE activity of peripheral nerves was barely inhibited by soman (28). A skeletal muscle study in rats indicated acute ultrastructural damage only at and adjacent to the motor endplates (29). Histologic and ultrastructural abnormalities in rat skeletal muscle fibers have been seen up to 3 weeks after soman exposure (29, 30).

The degenerative and regenerative skeletal muscle fiber changes in our monkeys were consistent with a 10-day duration. As postulated for cardiac myofibers, the lesions could be due to overstimulation, but few myofibers were affected in the skeletal muscle, compared with the heart. A peripheral effect at the neuromuscular junction seems more likely than does CNS overstimulation because only one to three adjacent myofibers were affected and only widely scattered foci were seen in each section of a muscle bundle. Affected myofiber foci were more numerous in mild, compared with minimal lesions, but only one to three adjacent myofibers were affected per focus. Neuromuscular junction overstimulation would rule out an adrenergic component because the neurotransmitter ACh is the local transmitter. Skeletal muscle changes were somewhat more extensive in the animals with convulsions, but lesions were present in nearly all animals, with and without convulsions, and there was no significant correlation between convulsions and muscle lesions. Perhaps, there is an association between muscular tremors and muscle lesions because all had tremors.

The lack of convulsions and lesions in group-2 animals was probably due to the lower doses of soman given, than those given to any of the group-1 animals. Only two of these were not further treated, and they received the lowest doses of soman (4.5 and 5.3  $\mu\text{g/kg}$ ), below the study's mean soman 50% lethal dose ( $\text{LD}_{50}$ ) of 6.5  $\mu\text{g/kg}$ . Nine of the 10 other group-2 animals received at least 1 to 2  $\text{LD}_{50}$ 's, but were treated.

In conclusion, one monkey without behavioral manifestations of convulsions had brain damage, indicating the need to monitor seizure activity by use of electroencephalography. All other brain lesions were found in monkeys that had convulsed. Since these lesions were in the hippocampus and amygdala, these adjacent areas should be given closer examination by removing them whole from half the brain and making sagittal sections.

Heart lesions and skeletal muscle lesions were not predictable by presence or absence of convulsions. The soman-induced pathogenesis of heart and muscle damage is unclear and should be evaluated in further studies of soman toxicity.

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